X-ray Reflectivity from Annexin Adsorbed onto Phospholipid Bilayers

E. Bitto, M. Li, A. M. Tikhonov, M. L. Schlossman, W. Cho (U. Illinois, Chicago)

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Beam line(s): **X19C**

It has been proposed that annexin I has two separate interaction sites that are involved in membrane binding and aggregation, respectively. To better understand the mechanism of annexin-I mediated membrane aggregation, we investigated the properties of the inducible secondary interaction site implicated in membrane aggregation. Phospholipid monolayers, containing POPC/POPE/POPS (2:5:2) were formed on an aqueous subphase buffer (10 mM HEPES-KOH, 0.1M KCl, pH 7.0) and compressed to a surface pressure of 34 dynes/cm. Annexin I (or V) was injected into the subphase. X-ray specular reflectivity measurements showed that the thickness of annexin I layer bound to the phospholipid monolayer was 31±2Å, indicating that annexin I binds membranes as a protein monomer of monolayer in the presence of 1 mM Ca^{2+}. Binding did not occur in the absence of Ca^{2+}. Similar results were shown for annexin V. These features for annexin-I can be seen clearly in the figure below. When combined with surface plasmon resonance measurements, these results support a hypothetical model of annexin-I mediated membrane aggregation, in which a laterally aggregated monolayer of membrane-bound annexin I directly interacts with a secondary membrane via its induced hydrophobic interaction site (to be published in *Biochemistry*).

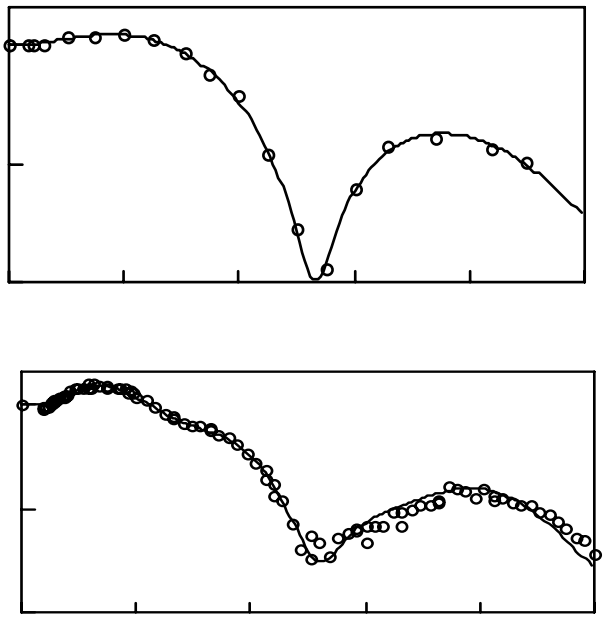

Fig. 1 X-ray reflectivity from annexin-I bound to phospholipid monolayers: (a) subphase with 1 mM Ca^{2+}, (b) subphase with annexin I (160μg) and 1 mM Ca^{2+}.